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DOI:

[10.1016/j.scr.2015.12.040](https://doi.org/10.1016/j.scr.2015.12.040)

Document Version

Publisher's PDF, also known as Version of record

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Citation for published version (APA):

Miere, C., Hewitson, H., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Stephenson, E., & Ilic, D. (2016). Generation of KCL029 research grade human embryonic stem cell line carrying a mutation in WAS gene. *Stem Cell Research*, 16(1), 189-191. <https://doi.org/10.1016/j.scr.2015.12.040>

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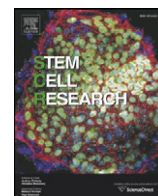
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Lab resource: Stem cell line

Generation of KCL029 research grade human embryonic stem cell line carrying a mutation in WAS gene



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ARTICLE INFO

Article history:

Received 31 December 2015

Accepted 31 December 2015

Available online 5 January 2016

ABSTRACT

The KCL029 human embryonic stem cell line was derived from an embryo donated for research that carried a c.814 T > C mutation in the WAS gene, which is linked to the Wiskott-Aldrich syndrome, a rare, inherited, X-linked, recessive disease characterized by immune dysregulation and microthrombocytopenia. The line is also carrier for a mutation p.N1152H in the gene encoding the cystic fibrosis transmembrane conductance regulator CFTR. The ICM was isolated using laser microsurgery and plated on γ -irradiated human foreskin fibroblasts. Both the derivation and cell line propagation were performed in an animal product-free environment. Pluripotent state and differentiation potential were confirmed by *in vitro* assays.

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1. Resource table

Name of stem cell line	KCL029
Institution	King's College London, London UK
Derivation team	Neli Kadeva, Victoria Wood, Glenda Cornwell, Stefano Codognotto, Emma Stephenson
Contact person and email	Dusko Ilic, email: dusko.ilic@kcl.ac.uk
Type of resource	Biological reagent: cell line
Sub-type	Human pluripotent stem cell line
Origin	Human embryo
Key marker expression	Pluripotent stem cell markers: NANOG, OCT4, TRA-1-60, TRA-1-81, alkaline phosphatase (AP) activity
Authentication	Identity and purity of line confirmed 1) Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. <i>Cytotherapy</i> . 14 (1), 122–128.doi: 10.3109/14653249.2011.623692 http://www.ncbi.nlm.nih.gov/pubmed/22029654 2) Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. <i>Nat. Protoc.</i> 7 (7), 1366–1381. doi: 10.1038/nprot.2012.080 http://www.ncbi.nlm.nih.gov/pubmed/22722371
Link to related literature (direct URL links and full references)	

(continued)

Information in public databases	KCL029 is a National Institutes of Health (NIH) registered hESC line NIH Registration Number: 0225 NIH Approval Number: NIHhESC-13-0225 http://grants.nih.gov/stem_cells/registry/current.htm?id=658
Ethics	The hESC line KCL029 is derived under license from the UK Human Fertilisation and Embryology Authority (research license numbers: R0075 and R0133) and also has local ethical approval (UK National Health Service Research Ethics Committee Reference: 06/Q0702/90). Informed consent was obtained from all subjects and the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the NIH Belmont Report. No financial inducements are offered for donation.

2. Resource details

Consent signed	Aug 12, 2009
Embryo thawed	Aug 23, 2009
UK Stem Cell Bank Deposit Approval	Dec 01, 2011
Sex	Reference: SCSC11-46
Grade	Male 46, XY
Disease status (Fig. 1)	Research
Karyotype (aCGH)	c.814 T > C mutation in the WAS gene and carrier for a mutation p.N1152H in the gene CFTR Duplication of approximately 0.23 Mb from the long arm of chromosome 3; 3q29(197,574,292–197,803,820)×3

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DNA fingerprint (Table 1)	Allele sizes (in bp) of 17 microsatellite markers specific for chromosomes 13, 18 and 21
Viability testing	Pass
Pluripotent markers (immunostaining) (Fig. 2)	NANOG, OCT4, TRA-1-60, TRA-1-81, AP activity
Three germ layers differentiation in vitro (immunostaining) (Fig. 3)	Endoderm: AFP (α-fetoprotein) Ectoderm: TUBB3 (tubulin, β3 class III) Mesoderm: ACTA2 (actin, α2, smooth muscle)
Sibling lines available	No

We generated KCL029 clinical grade hESC line following protocols, established previously (Ilic et al., 2012; Stephenson et al., 2012). The expression of the pluripotency markers was tested after freeze/thaw cycle (Fig. 2). Differentiation potential into three germ layers was verified in vitro (Fig. 3).

3. Materials and methods

3.1. Consenting process

We distribute Patient Information Sheet (PIS) and consent form to the in vitro fertilization (IVF) patients if they opted to donate to research embryos that were stored for 5 or 10 years. They mail signed consent back to us and that might be months after the PIS and consent were mailed to them. If in the meantime new versions of PIS/consent are implemented, we do not send these to the patients or ask them to re-sign; the whole process is done with the version that was given them initially. The PIS/consent documents (PGD-V.8) were created on Jul. 01, 2010. HFEA Code of Practice that was in effect at the time of document creation: Edition 8 – R.2 (<http://www.hfea.gov.uk/2999.html>). The donor couple signed the consent on Jan. 20, 2011. HFEA Code of Practice that was in effect at the time of donor signature: Edition 8 – R.2. HFEA Code of Practice Edition 8 – R.2 was in effect Apr. 07, 2010–Apr. 06, 2011.

3.2. Embryo culture and micromanipulation

Embryo culture and laser-assisted dissection of inner cell mass (ICM) were carried out as previously described in details (Ilic et al., 2012; Stephenson et al., 2012). The cellular area containing the ICM was then washed and transferred to plates containing mitotically inactivated human neonatal foreskin fibroblasts (HFF).

Table 1

Genotyping. Microsatellite markers specific for chromosomes 13, 18, 21, X and Y were amplified. The allele sizes in bp for markers on chromosomes 13, 18, and 21 are listed in the table.

Chr	Marker	Allele 1	Allele 2
13	D13S252	294	298
	D13S305	447	455
	D13S325	284	293
	D13S628	457	457
	D13S634	405	415
18	D18S386	352	375
	D18S390	360	372
	D18S391	217	225
	D18S535	482	482
	D18S819	400	408
	D18S976	476	480
	D18S978	207	211
	D21S11	248	248
21	D21S1409	212	212
	D21S1411	303	308
	D21S1435	184	188
	D21S1437	311	315

3.3. Cell culture

ICM plated on mitotically inactivated HFF were cultured as described (Ilic et al., 2012; Stephenson et al., 2012). TE cells were removed mechanically from outgrowth (Ilic et al., 2007; Ilic et al., 2010). hESC colonies were expanded and cryopreserved at the third passage.

3.4. Viability test

Straws with the earliest frozen passage (p.2–3) are thawed and new colonies are counted three days later. These colonies are then expanded up to passage 8, at which point cells were part frozen and part subjected to standard battery of tests (pluripotency markers, in vitro and in vivo differentiation capability, genetics, sterility, mycoplasma).

3.5. Pluripotency markers

Pluripotency was assessed using two different techniques: enzymatic activity assay [alkaline phosphatase (AP) assay] and immunostaining as described (Ilic et al., 2012; Stephenson et al., 2012).

3.6. Differentiation

Spontaneous differentiation into three germ layers was assessed in vitro and in vivo as described (Petrova et al., 2014; Ilic et al., 2012; Stephenson et al., 2012).

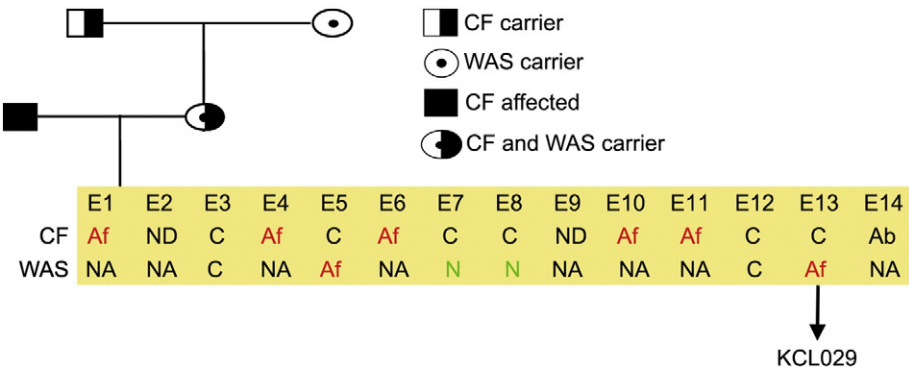


Fig. 1. Genetic pedigree tree. Both parents were carrying mutation in CFTR gene. Maternal CFTR mutation was p.N1152H and paternal ΔF508 and exon 2 deletion. In addition, the mother carried c.814 T > C mutation in WAS gene. The embryos were first genotyped for mutation in CFTR gene and then only normal and carrier embryos were assessed further for a mutation in WAS gene. Ab, abnormal; Af, affected; C, carrier; NA, non-applicable; ND, not determined.

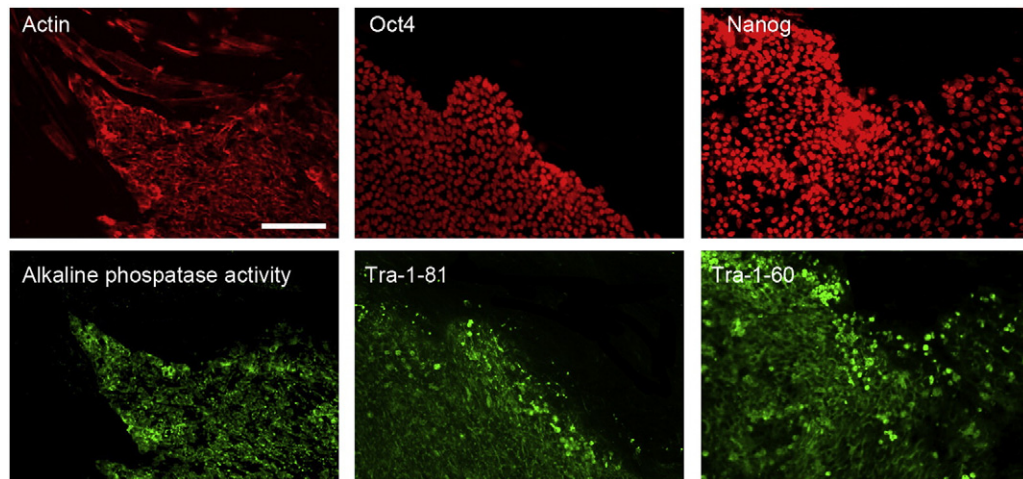


Fig. 2. Expression of pluripotency markers. Pluripotency is confirmed by immunostaining (Oct4, Nanog, TRA-1-60, TRA-1-81) and alkaline phosphatase (AP) activity assay. Actin stress fibers, visualized with rhodamine-phalloidin (red), are present in both feeders and hES cell colonies, whereas AP activity (green) is detected only in hES cells. Scale bar, 100 μ m.

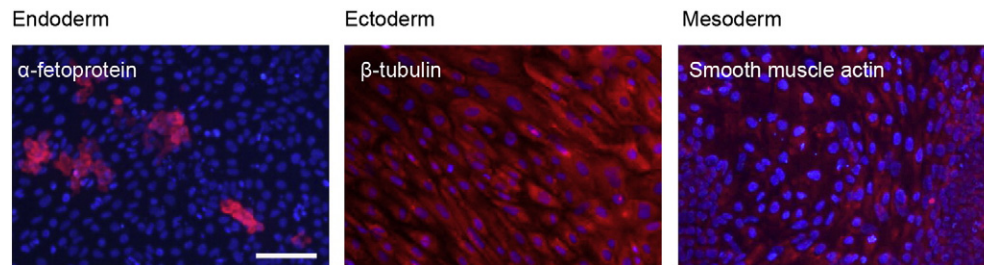


Fig. 3. Differentiation of three germ layers in vitro is confirmed by detection of markers: smooth muscle actin (red) for mesoderm, β -tubulin (red) for ectoderm and α -fetoprotein (red) for endoderm. Nuclei are visualized with Hoechst 33,342 (blue). Scale bar, 100 μ m.

3.7. Genotyping

DNA was extracted from hESC cultures using a Chemagen DNA extraction robot according to the manufacturer's instructions. Amplification of polymorphic microsatellite markers was carried out as described (Ilic et al., 2012). Allele sizes were recorded to give a unique fingerprint of each cell line.

3.8. Array comparative genomic hybridization (aCGH)

aCGH was performed as described in details (Ilic et al., 2012).

Author disclosure statement

There are no competing financial interests in this study.

Acknowledgments

This work was supported by the UK Medical Research Council grants G0701172 and G0801061. We thank Dr. Yacoub Khalaf, Director of the Assisted Conception Unit of Guy's and St Thomas' NHS Foundation

Trust and his staff for supporting the research program. We are especially indebted to Prof Peter Braude and to the patients who donated embryos.

References

- Ilic, D., Genbacev, O., Krtolica, A., 2007. Derivation of hESC from intact blastocysts. *Curr. Protoc. Stem Cell Biol.* (Chapter 1: Unit 1 A.2).
- Ilic, D., Caceres, E., Lu, S., Julian, P., Foulk, R., Krtolica, A., 2010. Effect of karyotype on successful human embryonic stem cell derivation. *Stem Cells Dev.* 19 (1), 39–46.
- Ilic, D., Stephenson, E., Wood, V., Jacquet, L., Stevenson, D., Petrova, A., Kadeva, N., Codognotto, S., Patel, H., Semple, M., Cornwell, G., Ogilvie, C., Braude, P., 2012. Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. *Cytotherapy* 14 (1), 122–128.
- Petrova, A., Celli, A., Jacquet, L., Dafou, D., Crumrine, D., Hupe, M., Arno, M., Hobbs, C., Cvoro, A., Karagiannis, P., Devito, L., Sun, R., Adame, L.C., Vaughan, R., McGrath, J.A., Mauro, T.M., Ilic, D., 2014. 3D In vitro model of a functional epidermal permeability barrier from human embryonic stem cells and induced pluripotent stem cells. *Stem Cell Rep.* 2 (5), 675–689.
- Stephenson, E., Jacquet, L., Miere, C., Wood, V., Kadeva, N., Cornwell, G., Codognotto, S., Dajani, Y., Braude, P., Ilic, D., 2012. Derivation and propagation of human embryonic stem cell lines from frozen embryos in an animal product-free environment. *Nat. Protoc.* 7 (7), 1366–1381.